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Antimicrobial Analysis of Traditional and Industrial Produced Vinegar

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Abstract

The vinegar produced from different locally grown fruits and industrial produced vinegar was evaluated to determine its antimicrobial properties. Agar well diffusion method was used for this analysis. The antimicrobial activities of both locally and industrially produced vinegar were identified using different microbial isolates which includes *Escherichia coli*, *Staphylococcus aureus* and *Candida sp* using different extracts of the vinegar.The results of the antimicrobial analysis showed that the vinegar exhibit different activities on the clinical isolates. Concentrations of 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml of the vinegar extracts were used. The aqueous and n-butanol extract of Vin A gave the highest zone of inhibition with a diameter of 19mm on *Staphylococcus aureus* at 500mg/ml concentration.The aqueous extract of Vin A gave diameter range of 15mm-19mm for *S. aureus* and 11mm-17mm for *Candida sp.* n-Butanol extract gave the range of 10mm-12mm for *E.coli*, 12mm-19mm on *S. aureus* and 7mm-15mm on*Candida sp.* The ethyl acetate of Vin A gave a diameter of 9mm and 10mm for *Candida sp.* The n-Butanol extract of Vin B gave the diameter of 10mm for *E.coli*at 500mg/ml, 6mm and 60mm for *S. aureus* and 9mm for *Candida sp.* the aqueous extract of Vin C gave diameter of ranges within 8mm-11mm for *E.coli*, and 10mm on c*Candida sp.* The n-Butanol extract gave the range of 7mm-10mm on *E.coli*, 8mm-12mm on *S. aureus* and 9-10mm on *Candida sp.* n-butanol extract of Vin D gave a diameter of 9mm for *E.coli*, 10mm for staph and a diameter range of 7-11mm for *Candida sp.* The ethylacetate of the extract gave 7mm and 8mm for *E.coli*, 9mm and 11mm for *Candida sp.* The vinegars analysed exhibited bactericidal, bacteriostatic and no activity on the clinical isolates.

INTRODUCTION

Vinegar is an ancient fermented food present and consumed by man since Babylonians period. Historically, speaking, vinegar has a history of more than 6000 BC. Vinegar is derived from French word 'Vin' meaning wine and the word 'aigre' meaning sour. The Holy Bible mentions it and it has been used as a therapeutic agent [1]. There have been many therapeutic benefits associated with the consumption of synthetic vinegar like decreasing the cholesterol and hypertension level.

Vinegar is defined as "a liquid fit for human consumption that is produced from the appropriate raw materials of agricultural origin containing starch, sugars, or starch and sugars by the process which involves double fermentation, alcoholic and acetous, containing a specified amount of acetic acid" [2]. Traditionally, it has been regarded as a natural food. Not only this, it acts as a preservative because of its acetic acid content and consequently, lowers the pH of food and hence, help in preservation [3]. Vinegar has been reported to retard microbial growth and improve the sensory properties of foods.

Productions of vinegar are mostly carried at home scale/ cottage industry using natural fermentation. With respect to cider vinegar, the vinegar made from apple, the acetification is also carried out naturally but is a slow process [4].

Vinegar is a solution of acetic acid produced by a two-step bioprocess. In the first step, fermentable sugars are transformed into ethanol by the action of yeast. In the second step, Acetic acid bacteria (AAB) convert the ethanol into acetic acid in an aerobic process [5]. Acetic acid is a carboxylic acid with antibacterial and antifungal properties found in Vinegar. During the fermentation process, this acid slowly grows to become a non-toxic slime that some people call the "mother". The "mother" is the dark, cloudy substance in unfiltered Vinegar that forms from naturally occurring pectin and residues. It typically appears as molecules of

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protein connected in strand-like chains or appears to be webbed form. Its presence in the vinegar is a confirmation that the best part of the substrate is intact and that the vinegar is of the highest quality [5]. Microbial species involved in fermentations includes yeast, molds, lactic acid bacteria (LAB) and acetic acid bacteria (AAB). The main microorganisms involved in vinegar production are mainly yeasts and AAB. The former being responsible for the alcoholic fermentation and the latter needed for the acetic acid production [6-8]. For cider production, the strains commonly used belongs to the species *Saccharomyces cerevisiae* or *Saccharomyces bayanus* and the choice of yeast strain to be used as starters could have a high impact on the flavor profile of fermented beverages [9]. The results from the study revealed that during fermentation of apple juice, the rate and content of, sugars, tannins, esters, methanol, ethanol and volatile acids were some of the quality characteristics that could be affected by the specific yeast strain. Acetic acid bacteria are present in the environment and in the raw material, but cannot grow during alcoholic fermentation because of the anaerobic conditions but when the alcoholic liquid is exposed to oxygen, the acetic acid bacteria starts to grow on the surface [10]. According to the reports of Du Toit and Pretorius (2002), the most AAB growth was observed between pH 5.4 - 6.3, but growth can also be seen at pH values lower than 4 [11]. Yamada and Yukphan. (2008) studies showed that the presence of AAB was usually found in substrates containing sugar and/or ethanol [12]. These substrates include fruits, food, flowers and fermented beverages, such as fruit juices, cocoa, wine, beer, cider, and vinegar. Although a variety of bacteria can produce acetic acid, mostly members of *Acetobacter* (Gluconoacetobacter) are used for commercial purposes, typically the aerobic bacterium *A. aceti* at 27°C - 37°C [4; 13-15]. The antimicrobial activity occurs as a result of the diffusion of the acidic molecules into microbial cells until equilibrium is reached, in accordance with the pH gradient, causing membrane disruption, inhibition of essential metabolic reactions, stress on intracellular pH homeostasis and accumulation of toxic anions and ultimately death of microbial cells [16].

The aim of this research work is to evaluate the antimicrobial properties of the traditional and industrial produced vinegar

MATERIALS AND METHODS

The local and industrial vinegar samples produced using the method by Ezemba *et al* (2021) was procured at Chychy Gilgal Ltd laboratory and consultancy services, Ichida [4]. The vinegar samples procured were labeled Vin A, Vin B and Vin C based on the raw materials used.

Vin A = vinegar from combination of lemon, lime, orange, grape (all with the peel)
Vin B= vinegar from combination of green and red apple with the peels bought from Awka
Vin C= vinegar from vinegar from pawpaw, jackfruit, pineapple with peel and oranges
Vin D= Bragg (organic) raw unfiltered apple cider vinegar with mother

DETERMINATION OF ANTIMICROBIAL ACTIVITY USING AGAR WELL DIFFUSION METHOD

The vinegar samples were fractionated into four fractions; n-hexane, ethyl acetate, n-butanol and aqueous fraction using solvent extraction All the fractions of the vinegar sample were used to test for the antimicrobial activity. The agar diffusion method was used on a solid sterile nutrient medium (Müeller Hinton agar-MHA) to test the antibacterial activity of apple cider vinegar on selected bacterial cultures.

Cultures of *E. coli* and *S. aureus* were grown in nutrient agar whereas *C. albicans* was grown in Sabouraud Dextrose medium. All cultures were cultivated and incubated at 37 °C for 24 h overnight prior to use. All microbial cultures were adjusted to 0.5 McFarland's standard (1.5×10^8 CFU/ml) of each organism used in experiments. Each microbe was swabbed evenly onto plates containing MHA and SDA (for fungi).

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A cork borer of 6mm was used to bore holes on the agar plates that have already been seeded with the various organisms. Using micropipette 0.1mls of the vinegar samples was poured into in the holes bored. The antimicrobial drug ciprofloxacin was used as a control. This method's working principle is based on the fact that the antimicrobial agent diffuses in the medium and expands radially, with its concentration decreasing as distance from the edges increases [17]. If the bacteria are susceptible to the action of the tested antimicrobial agent, it will not grow in the zone of its action. Therefore, after incubation, the zones of absence of growth are observed around the cylinder, so called the inhibition zones. The zones of growth inhibition were measured using a ruler and pair of dividers and the sensitivity of the bacterial strain to the tested vinegar sample were determined. Petri plates were incubated for 24 hours at a temperature of 37°C and 72 hrs for fungi.

TYPE OF ANTIMICROBIAL ACTIVITY

This was carried out to determine the analysed vinegar antimicrobial activity (bactericidal/ fungicidal or bacteriostatic/ fungistatic activity). A small piece of agar was taken from the inhibition zone and added to the nutrient broth. Incubation was carried out 24h at 37°C for bacteria and 3days for fungi. If, after incubation, there is cloudy broth, it is considered that the vinegar is bacteriostatic/ fungistatic, while if, after incubation, the broth remains clear, the effect of vinegar is bactericidal/ fungicidal [17]. **RESULTS**

ANTI-MICROBIAL ACTIVITY OF THE VARIOUS VINEGAR SAMPLES ON SELECTED CLINICAL ISOLATES

This analysis was carried out to determine the effect of the vinegar on some pathogenic organisms. The vinegar samples underwent an extraction process using some solvents which includes n-butanol, n-hexane and ethyl acetate. The extracts were now used to test for the antimicrobial activities.

The vinegar samples used includes

Vin A = vinegar from combination of lemon, lime, orange, grape (all with the peel)
Vin B= vinegar from combination of green and red apple with the peels bought from Awka
Vin C= vinegar from vinegar from pawpaw, jackfruit, pineapple with peel and oranges
Vin D= Bragg (organic) raw unfiltered apple cider vinegar with mother

From the result presented in Table 1, The aqueous extract of Vin A showed no inhibitory effect on *Escherichia coli* at the different concentrations but for *Staphylococcus aureus* gave an inhibition diameter of 19mm at 500mg/ml and 16mm for 250mg/ml and 15mm for 125mg/ml and 9mm for 62.5mg/ml while for *Candida sp*, at 500mg/ml, it gave 17mm, at 250mg/ml., it gave 15mm, 12mm at 125mg/ml and 11mm at 62.5mg/ml. The n-butanol extract showed that at concentration of 125mg/ml it had the highest zone of inhibition with diameter of 12mm for *E.coli* and highest for *Staphylococcus aureus* at 500mg/ml with 19mm and lowest with 12mm at the concentration of 62.5mg/ml while for *Candida*, the highest zone was at the concentration of 15mm at 500mg/ml and lowest at 62.5mg/ml with 7mm.There was no activity for n-hexane at all concentration on all the isolates. The ethyl acetate extract was only active on *Candida* with the highest zone of inhibition being at 500mg/ml with 12mm and the lowest as 125mg/ml with 9mm.

Aqueous extract of sample B had no activity on any of the isolates at all the concentrations. The n-butanol had activity on *E. coli* at 500mg/ml with an inhibition diameter of 10mm and *Staphylococcus* with 8mm and Candida at 9mm at the same concentration. The extract had activity on Staphylococcus at concentration of 62.5mg/ml with inhibition diameter of 12mm and *Candida* at concentration of 250mg/ml with inhibition diameter of 9mm. N-hexane and ethyl acetate has no activity on any of the isolates at all the concentrations.

For Vin C, the aqueous extract has activity on only the *E. coli* (11mm) and *Candida* (10mm) but no

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activity on *Staphylococcus aureus* at the concentration of 500mg/ml. At 250mg/ml, the extract was only active o n *E.coli* and all other concentrations didn't have any activity on any of the organisms. The n-butanol had activity on the three isolates. The extract had activity on the *E. coli* with 500mg/ml with 10mm as the inhibition diameter and lowest of 7mm at 125mg/ml. On *Staphylococcus*, the 500mg/ml gave an inhibition diameter of 12mm and the lowest at 250mg/ml with inhibition diameter of 8mm. On *Candida*, the highest zone of inhibition was found at 500mg/ml with an inhibition diameter of 15mm while it is lowest at 8mm in the concentration of 125mg/ml. N-hexane was active on only *Candida* at the concentration of 500mg/ml but was no active on any other isolates at any other concentrations. The ethyl acetate had no activity on any of the isolates at any of the concentrations.

In Vin D, the aqueous and n-hexane extract had no activity but the n- butanol had activity on the *E.coli* at concentration of 500mg/ml with inhibition diameter of 9.5mm and *Staphylococcus* with inhibition diameter of 10mm and *Candida sp* at 11mm. *Candida sp* at 250mg//ml and 125mg/ml is sensitive to extract with diameter of 8mm and at 62.5mg/ml with inhibition diameter of 7mm. *E.coli* was sensitive to the ethyl acetate extract at concentration of 500mg/ml with inhibition diameter of 8mm and 9mm for the concentration at 250mg/ml . *Candida sp* is sensitive at 500mg/ml with inhibition diameter of 11mm and at 250mg/l with ihhibition diameter of 9mm.

Ciprofloxacin acted as the positive control with a diameter of 17mm and 16mm for *Escherichia coli and Staphylococcusaureus* respectively. Distilled water serves as the negative control with no zone of inhibition.

The vinegar activity was carried out to determine the type off effect that the vinegars have on the isolates. Only those that showed activity were subjected to ascertain the type of activity expressed. Aqueous extract of Vin A, exhibited a bactericidal effect at concentration of 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ for both *Staphylococcus* and *Candida*. The n-butanol extract exhibited bactericidal action on *E. coli* at concentrations of 500mgml, 250mg/ml and 125.5mg/ml, on *Staphylococcus* and *Candida* at concentration of 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml. Ethyl acetate extract exhibited bactericidal action on *Candida sp* at concentrations of 500mg/ml, 125mg/ml and 62.5mg/ml.

N-butanol extract of Vin B exhibited bactericical effect on the three isolates at concentration of 500mg/ml and bacteriostatic effect on *Candida* at concentration of 250mg/ml.

N-butanol extract of sample C exhibited bactericidal action on *E.coli* isolates at concentrations of 5000mg/ml, 250mg/ml and 125mg/ml and the *Staphylococcus* at concentrations of 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml and exhibited bacteriostatic activity on *Candida* at concentrations of 500mg/ml, 250mg/ml, 125mg/ml. the aqueous extract exhibited bacteriostatic activity on *E.coli* at concentrations 500mg/ml and 250mg/ml.

N-butanol extract of sample D gave bactericidal effect on *Candida* at concentration so 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml. The ethyl acetate of sample gave a bactericidal effect on *E. coli* at concentration of 500mg/ml and bacteriostatic effect on concentration of 250mg/ml and bacteriostatic effect on *Candida* at concentrations 500mg/ml and 250mg/ml.

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Table 1: Anti-microbial activity of the various vinegar samples on selected clinical isolates

Extract	Extract concentration (mg/ml)	Zones of inhibition (mm)		
		Escherichia coli	*Staphylococcusaureus*	*Candida sp,*
Vin A				
Aqueous	500	-	19±2.0	17±1.5
	250	-	16±1.0	15±0.1
	125	-	15±3.0	12±0.5
	62.5	-	9±0.5	11±1.3
n-butanol	500	10±1.0	19±1.0	15±1.8
	250	8±0.5	18±2.0	10±0.9
	125	12±0.1	18±0.3	8±1.0
	62.5	-	12±0.7	7±0.1
n-hexane	500	-	-	-
	250	-	-	-
	125	-	-	-
	62.5	-	-	-
Ethyl acetate	500	-	-	12±0.5
	250	-	-	-
	125	-	-	9±0.1
	62.5	-	-	10±0.2
Vin B				
Aqueous	500	-	-	-
	250	-	-	-
	125	-	-	-
	62.5	-	-	-
n-butanol	500	10±1.0	8±0.8	9±1.0
	250	-	-	9±1.5
	125	-	-	-
	62.5	-	12±1.4	-
n-hexane	500	-	-	-
	250	-	-	-
	125	-	-	-
	62.5	-	-	-
Ethyl acetate	500	-	-	-

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	250	-	-	-
	125	-	-	-
	62.5	-	-	-
Vin C				
Aqueous	500	11±0.5	-	10±1.2
	250	8±0.5	-	-
	125	-	-	-
	62.5	-	-	-
n-butanol	500	10±0.5	12±0.3	9±0.6
	250	8±0.3	8±1.2	9±0.5
	125	7±0.5	8±0.7	-
	62.5	-	10±0.5	-
n-hexane	500	-	-	10±1.0
	250	-	-	-
	125	-	-	-
	62.5	-	-	-
Ethyl acetate	500	-	-	-
	250	-	-	-
	125	-	-	-
	62.5	-	-	-
Vin D				
Aqueous	500	-	-	-
	250	-	-	-
	125	-	-	-
	62.5	-	-	-
n-butanol	500	9.5±0.5	10±1.1	11±0.2
	250	-	-	8±0.1
	125	-	-	8±0.5
	62.5	-	-	7±0.5
n-hexane	500	-	-	-
	250	-	-	-
	125	-	-	-
	62.5	-	-	-
Ethyl acetate	500	8±0.8	-	11±1.0

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	250	7±0.2	-	9±1.5
	125	-	-	-
	62.5	-	-	-
Ciprofloxacin	30µg	19	18	
Distilled water		-	-	

Key : - no zone of inhibition
Vin A = combination of lemon, lime, orange, grape (all with the peel)
Vin B= combination of green and red apple bought from Awka
Vin C=vinegar from pawpaw, jackfruit, pineapple with peel and oranges
Vin D= Bragg (organic) raw unfiltered apple cider vinegar with mother.
Vin A and C are locally Produced Vinegar from different fruit mix
Vin B is locally Produced Vinegar from Apple purchased in the local market
Vin D is sample of industrially produced vinegar

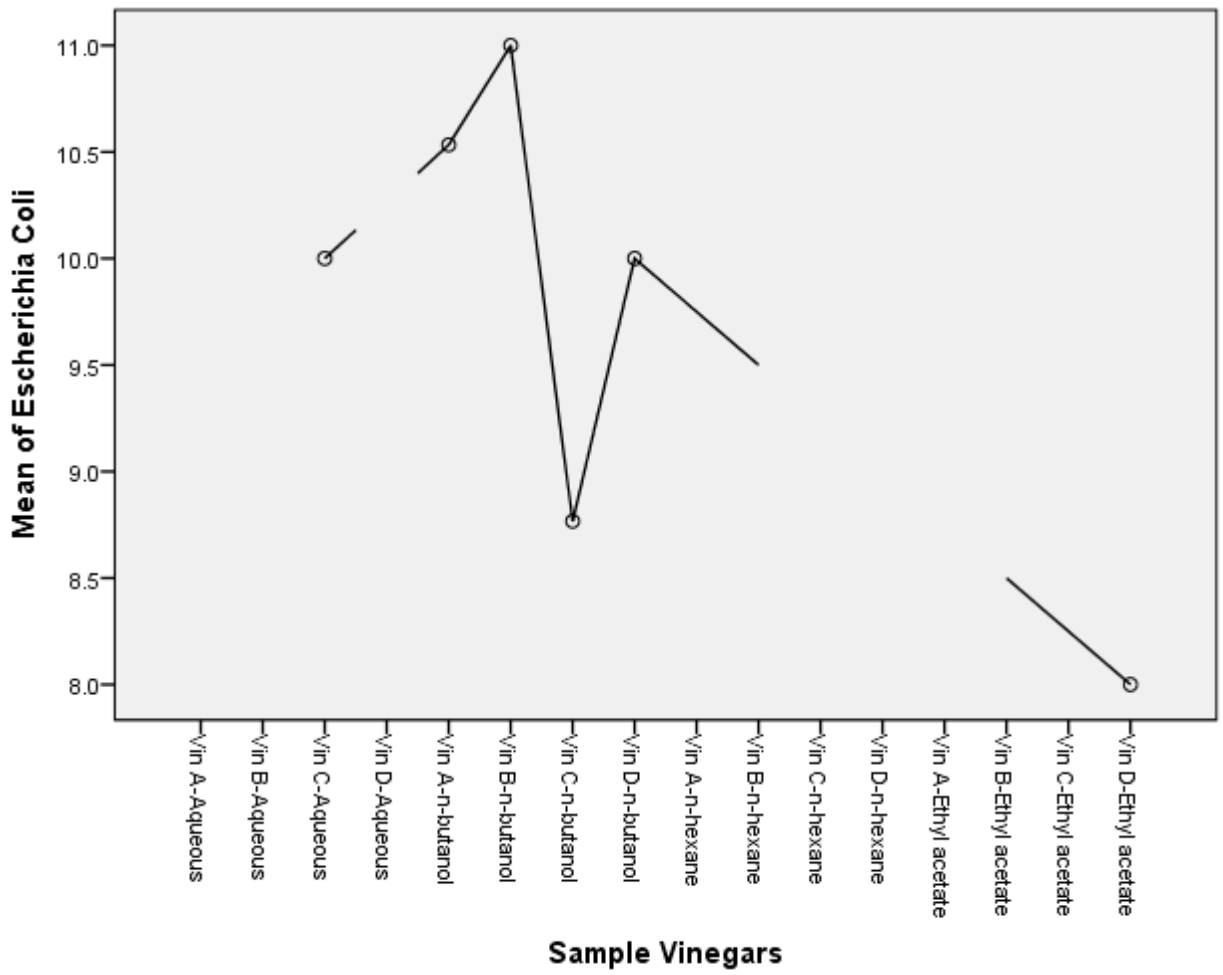

Fig 1: Mean plot of the zone of inhibition created by the vinegar samples on *Escherichia coli*

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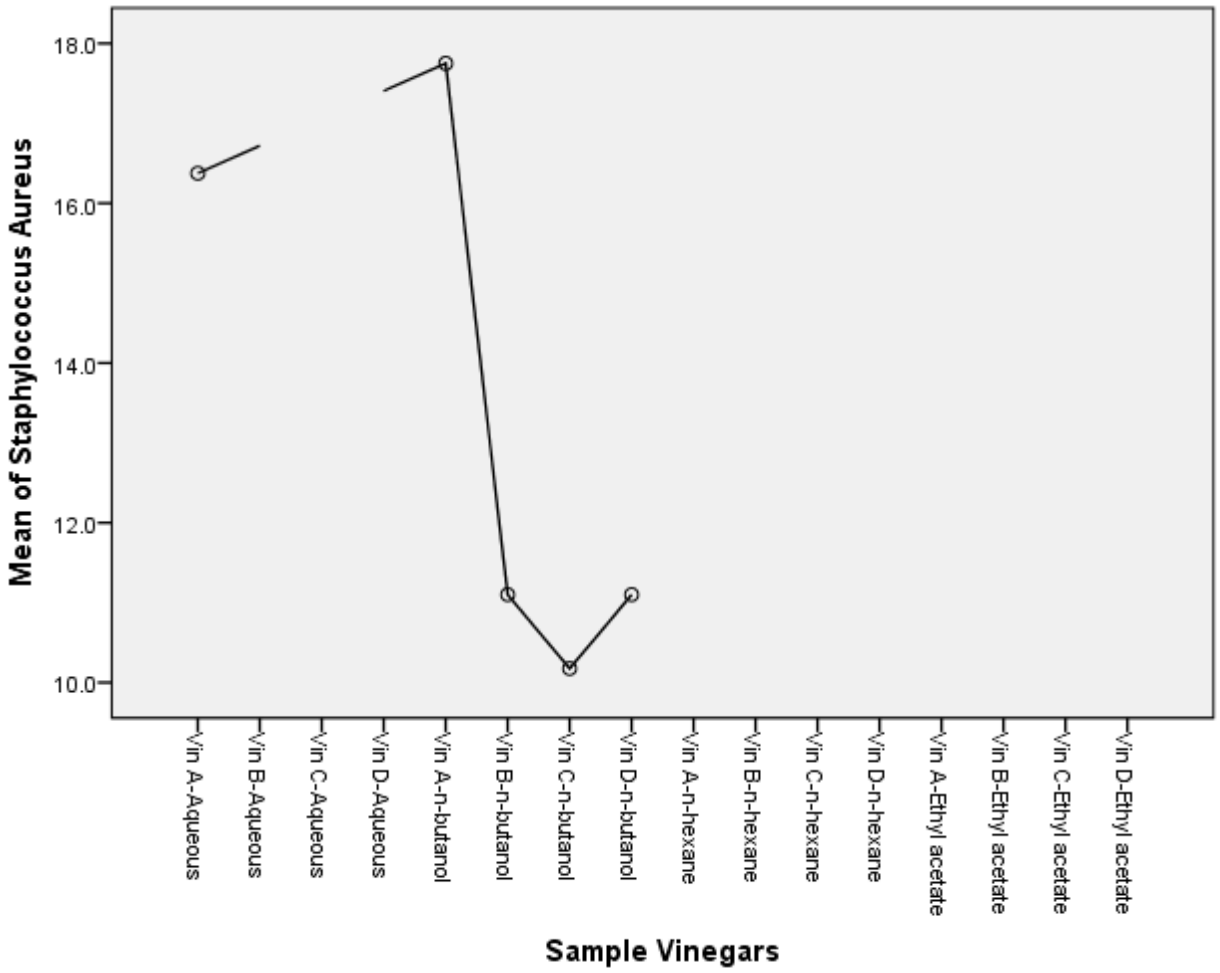

Fig 2: Mean plot of the zone of inhibition created by the vinegar samples on *Staphylococcus aureus*

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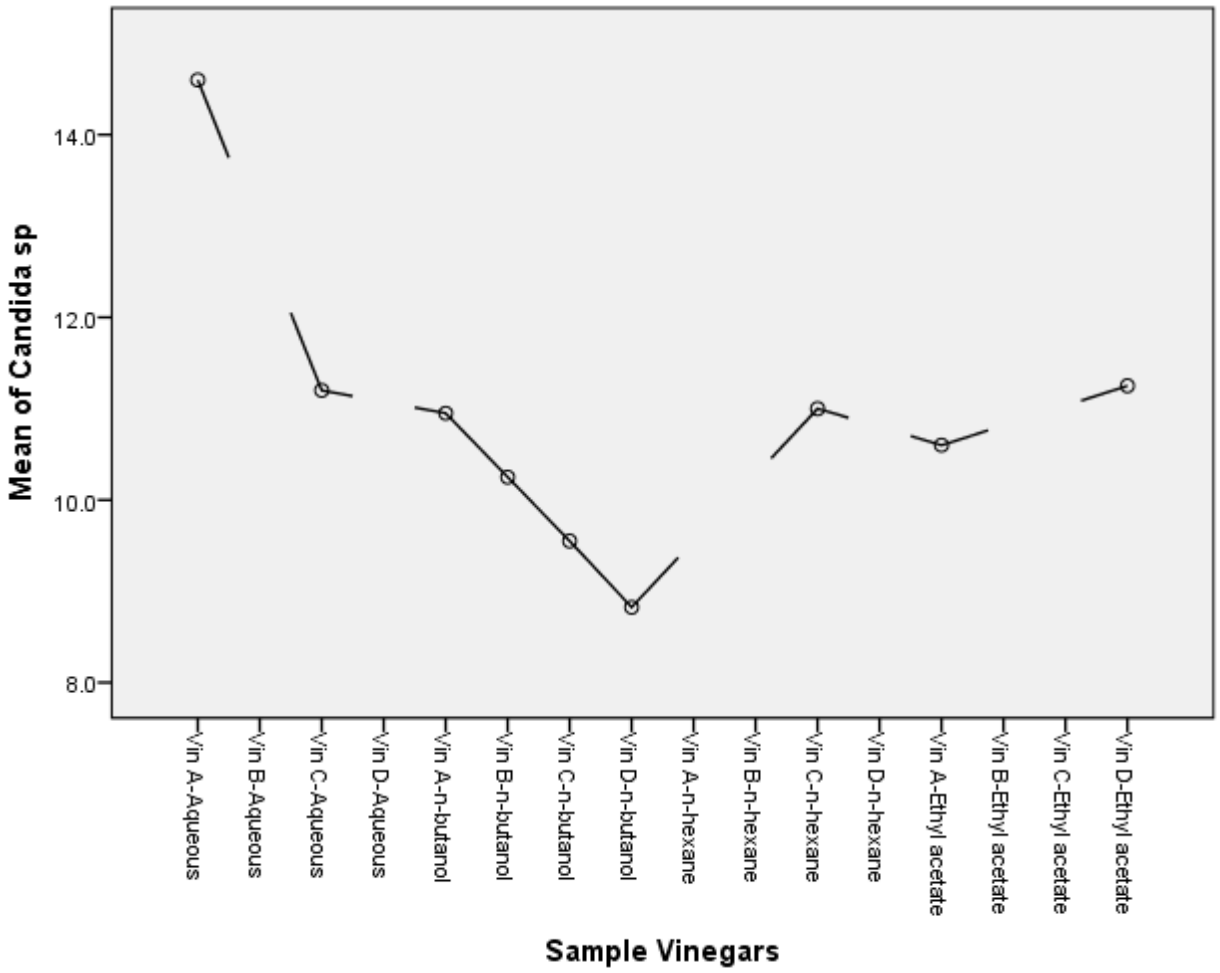

Fig 3: Mean plot of the zone of inhibition created by the vinegar samples on *Candida sp*

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Table 2: Determination of the type of antimicrobial activity of vinegar

Extract	Extract concentration (mg/ml)	Zones of inhibition (mm)		
		Escherichia coli	*Staphylococcusaureus*	*Candida sp,*
Vin **A**				
Aqueous	500	ND	-	-
	250	ND	-	-
	125	ND	-	-
	62.5	ND	-	-
n-butanol	500	-	-	-
	250	-	-	-
	125	-	-	-
	62.5	ND	-	-
n-hexane	500	ND	ND	ND
	250	ND	ND	ND
	125	ND	ND	ND
	62.5	ND	ND	ND
Ethyl acetate	500	ND	ND	-
	250	ND	ND	ND
	125	ND	ND	-
	62.5	ND	ND	-
Vin B				
Aqueous	500	ND	ND	ND
	250	ND	ND	ND
	125	ND	ND	ND
	62.5	ND	ND	ND
n-butanol	500	-	-	-
	250	ND	ND	+
	125	ND	ND	ND
	62.5	ND	-	ND
n-hexane	500	ND	ND	ND
	250	ND	ND	ND
	125	ND	ND	ND
	62.5	ND	ND	ND
Ethyl acetate	500	ND	ND	ND

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	250	ND	ND	ND
	125	ND	ND	ND
	62.5	ND	ND	ND
Vin C				
Aqueous	500	+	ND	-
	250	+	ND	ND
	125	ND	ND	ND
	62.5	ND	ND	ND
n-butanol	500	-	-	+
	250	-	-	+
	125	-	-	ND
	62.5	ND	-	ND
n-hexane	500	ND	ND	-
	250	ND	ND	ND
	125	ND	ND	ND
	62.5	ND	ND	ND
Ethyl acetate	500	ND	ND	ND
	250	ND	ND	ND
	125	ND	ND	ND
	62.5	ND	ND	ND
Vin D				
Aqueous	500	ND	ND	ND
	250	ND	ND	ND
	125	ND	ND	ND
	62.5	ND	ND	ND
n-butanol	500	-	+	-
	250	ND	ND	-
	125	ND	ND	-
	62.5	ND	ND	-
n-hexane	500	ND	ND	ND
	250	ND	ND	ND
	125	ND	ND	ND
	62.5	ND	ND	ND

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Ethyl acetate	500	-	ND	+
	250	+	ND	+
	125	ND	ND	ND
	62.5	ND	ND	ND

Key : - no microbial growth, + microbial growth, ND- not determined

Vin A = combination of lemon, lime, orange, grape (all with the peel)
Vin B= combination of green and red apple bought from Awka
Vin C=vinegar from pawpaw, jackfruit, pineapple with peel and oranges
Vin D= Bragg (organic) raw unfiltered apple cider vinegar with mother.
Vin A and C are locally Produced Vinegar from different fruit mix
Vin B is locally Produced Vinegar from Apple purchased in the local market
Vin D is sample of industrially produced vinegar

DISCUSSIONS

Antibiotic resistance is rapidly becoming a major worldwide problem. There has been a steady increase in the number of pathogens that show multiple drug resistance, hence the need to explore more options in therapeutic handling of microbial infections. The antimicrobial analysis was carried out to determine the effect of the vinegars on certain pathogenic microorganisms which includes, *Staphylococcus aureus, Echerichia coli and Candida sp.* Solvent extraction was carried out to accentuate the active ingredients in the samples based on their polarities. In this study, the vinegar samples were partitioned using different solvent with different levels of polarity. The n-butanol is a polar solvent, the ethyl acetate is the moderately polar solvent and n-hexane is a non polar solvent and what remains after the partitioning is regarded as the aqueous. The different isolates react differently to the various partitions as can be seen in the results.

The extracts were tested on the pathogenic organisms to determine the effect of the extract on the organisms. Each of the vinegar was extracted using ethylacetate, n-butanol, n-hexane and the aqueous extract was also tested.

From the result obtained, aqueous extract of Vin A had an effect on *Staphylococcus aureus* and *Candida sp* at all concentrations but n-butanol extract was effective in all concentrations except 62.5mg/ml. n-hexane extract had no effect on the organisms. Ethylacetate extract was effective on only Candida *sp* at concentrations of 500,125 and 62.5mg/ml. Ethylacetate, n-hexane and aqueous extract of Vin B had no effect on the organisms but n-butanol was effective on *Staphylococcus aureus, Echerichia coli and Candida sp* at 500mg/ml and on *S.aureus*at 62.5mg/ml. Aqueous extract of Vin C was effective on *E.coli* at concentrations of 500 and 250mg/ml and *Candida sp* at 500mg/ml but has no effect on *S.aureus*. n-butanol was effective at all concentrations on *S.aureus*. It was effective at all concentrations on *E.coli* except 62.5mg/ml and *Candida sp* except at 125 and 62.5mg/ml. n-hexane was found effective only on *Candida sp* at 500mg/ml but not in any other concentration. Ethylacetate extract of Vin C had no effect on any of the organism. N-butanol extract of Vin D had effect on *Candida sp* at all concentrations but *E.coli* and *S.aureus* at 500mg/ml. Ethyl acetate extract was effective on *E.coli* and *Candida sp* at 500 and 250mg/ml. This work is similar to the work of Tumane *et al.* (2018) who reported that apple cider vinegar has also been reported to posess potent antibacterial activity against gram positive and negartive bacterial strains [18]. From the results on this work it can be said that the aqueous and n-butanol extracts of the vinegar gave higher antimicrobial effects than the other extracts analysed. Since n-butanol and water (aqueous) are polar solvents, it could mean that polar componenets of the vinegar have a have a

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greater antimicrobial activity than the other components.

This result agrees with the work of Chan *et al.* (2012) who recently reported that Matang wood vinegar displayed potent antibacterial activity against the strains of Gram-positive *Bacillus cereus, Micrococcus luteus* and *Staphylococcus aureus*, and Gram-negative *Escherichia coli, Salmonella typhi,* and *Pseudomonas aeruginosa* [19]. Chang and Fang. (2007) also reported antibacterial effect of rice vinegar on *E.coli* [20]. The inhibition of microbial growth increases by lowering pH of the media, and most microorganisms are susceptible to antimicrobial effects in the presence of organic acids. This phenomenon is due to the hydrophobic feature of most organic acids, which allows free diffusion of the protonized form through cell membrane. This diffusion process takes place spontaneously due to pH and osmolarity gradients that exist between the inner and outer sides of the cell. The intracellular pH is higher than the extracellular, and the acid undergoes dissociation as soon as it enters the cytoplasm and then decreases the intracellular pH by releasing the proton. To counter the decrease of cytoplasmic pH, resulting from the ionization of the entered acid, the cell allocates the main part of its energy content to eliminate these newly formed protons which results in slower growth kinetics [21]. According to Hassan *et al.* (2015) acetic acid shows strongest inhibition of fungal growth among other organic acids [21]. Earlier reports showed that the major components of organic acids and phenolics in wood vinegar can inhibit pathogenic fungi and bacteria [22-25]. In addition, in gram negative bacteria, the outer membrane acts as a selective permeability barrier in limiting or preventing the entry of many unnecessary or harmful chemical compounds into the bacterial cell [26]. According to the work of Yang *et al.* (2016) the phenolics and minor organic acids contribute to the antibacterial activities of the wood vinegar from Litchi chinensis [27]. Apple cider vinegar is a commonly prescribed antifungal agent in folk medicine for treatment of fungal skin, ear and vaginal infections [28]. The antifungal activity of apple cider vinegar might be attributed to its malic acid, acetic acid contents or to other non-identified ingredients. The mechanism of inhibition fungi growth by organic acids is generally not considered a pH phenomenon. It is known that, growth and morphology of fungi are influenced by the pH of media [29]. Organic acids resulting a decreasing in pH value, this may influence the growth by acidifying the cell, which will consume a great amount of energy to maintain the intracellular pH homeostasis [21]. Acetic acid in vinegar is a particularly effective antimicrobial, because at a relatively high pH (pH 4.7=pKa of acetic acid) it exists primarily in its undissociated form and can enter the cell [30]. The properties of undissociated organic acids such as fat-solubility and neutral charge enable them to passively diffuse through the cell membrane of the target microorganism; in the cytoplasm the higher intracellular pH causes the acid to become dissociated, producing primarily hydrogen ions (H^+), but also acetate ions (CH_3COO^-). These ions are toxic to the cell and interfere with cellular processes such as enzyme activity, DNA replication and transcription, and protein expression, therefore effecting the normal growth of the microorganism [31-32]. Antibacterial mechanisms of vinegar are primarily due to its acetic acid content [33]. Other explanations have also been proposed including the membrane disruption, the interruption of metabolic reactions, and the accumulation of toxic anions [34]. The antimicrobial activity of organic acid is influenced by the target bacterial strains, temperature, pH, acid concentration and ionic strength [35].

The anti-microbial agents used to treat gram negative infections such as β-lactams, fluroquinolones, sulfamethaoxathoxazole and trimethroprin are becoming increasingly ineffective. Furthermore, antibiotic action itself can be problematic in terms of cell membrane permeability, intracellular inactivation and the inability to reach intracellular structures in which organisms can hide. Alternative supplementation that can combat a plethora of microbes without concurrent side effects would be of significant

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healthcare interest as the discovery of effective new antibiotic has been slow but should be a global priority [27]. Vinegar has been very helpful in cleaning and treating nail fungus, head lice, warts and ear infections. Consumers normally prefer the use natural preservatives for inhibiting the growth of food borne pathogens in food. Several problems might be encountered with antifungal drugs, first; resistance; as fungi may become resistant to antifungal drugs due to target gene mutations, enzyme modification or to development of pump system that expels the drug out of the fungal cell [36]. second; toxicity; as antifungal drugs can cause systemic toxicities as hepatotoxicity and nephrotoxicity. These problems necessitate searching for safer effective remedies with known antifungal activity [28].

Aqueous extract of Vin A, exhibited a bactericidal effect at concentration of 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ for both *Staphylococcus aureus* and *Candida sp*. The n-butanol extract exhibited bactericidal action on *E. coli* at concentrations of 500mgml, 250mg/ml and 125.5mg/ml, on*S.aureus* and *Candida sp*at concentration of 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml. Ethyl acetate extract exhibited bactericidal action on *Candida sp* at concentrations of 500mg/ml, 125mg/ml and 62.5mg/ml.

N-butanol extract of Vin B exhibited bactericical effect on the three isolates at concentration of 500mg/ml and bacteriostatic effect on *Candida* at concentration of 250mg/ml.

N-butanol extract of Vin C exhibited bactericidal action on *E.coli* isolates at concentrations of 5000mg/ml, 250mg/ml and 125mg/ml and the *S.aureus*at concentrations of 500mg/ml, 250mg/ml, 125mg/ml 62.5mg/ml and exhibited bacteriostatic activity on *Candida sp* at concentrations of 500mg/ml, 250mg/ml, 125mg/ml. The aqueous extract exhibited bacteriostatic activity on *E.coli* at concentrations 500mg/ml and 250mg/ml.

N-butanol extract of Vin D gave bactericidal effect on *Candida* at concentration so 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml. The ethyl acetate of sample gave a bactericidal effect on *E. coli* at concentration of 500mg/ml and bacteriostatic effect on concentration of 250mg/ml and bacteriostatic effect on *Candida* at concentrations 500mg/ml and 250mg/ml.

This result agree with the work of Kalaba*et al.* (2019) apple vinegar displayed 100.00% bactericidal activity against *S. aureus*, and 100.00% bacteriostatic activity against *E. coli* [17]. Whether vinegars with or without their peels will have bactericidal or bacteriostatic activity depends to a large extent on the acetic acid concentration in the vinegar, the incubation time and the number of surviving bacteria. The bactericidal activity of apple vinegar increases with temperature [17]. From the results, it can be seen that the vinegars showed antimicrobial properties hence, its use should be encouraged.

References

1. Solieri L and Giudici P. (2009). Vinegars of the world.*Springer-Verlag*. 17 –39.
2. Pooja S. and Soumitra. B. (2013). Optimization of Process Parameters for Vinegar Production using Banana Fermentation. *International Journal of Research in Engineering and Technology* 501 – 51
3. Joshi, V.K. and Thakur, N.S (2000). Vinegar: composition and production. In: Verma, L.R., Joshi VK (eds) post havest technology of fruits and vegetables indus publishing, New Dehli. Pp1128–1170.
4. Ezemba A.S, Osuala O.J., Orji M.U., Ezemba C.C., Anaukwu C (2021). Production and comparative physicochemical analysis of vinegar from locally grown fruits in Nigeria and industrial produced vinegar. *American Journal of Microbiological Research*, **9**(1): 25–33.
5. Bhat, S.V, Akhtar,R.and Amin, T (2014).An Overview on the Biological Production of Vinegar. *International journal of fermented foods,* **3**(2):139–155.
6. Nanda, K., Taniguchi, M., Ujike, S., Ishihara, N., Mori, H., Ono, H. and Murooka, Y. (2001). Characterization of acetic acid bacteria in traditional acetic acid fermentation of rice

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DOI: http://doi.org/10.5281/zenodo.5716127

vinegar (komesu) and unpolished rice vinegar (kurosu) produced in Japan. *Applied and Environmental Microbiology,* **67**: 986 –990.

7. Haruta S, Ueno S, Egawa I, Hashiguchi K, Fujii A, Nagano M, Ishii M. and Igarashi Y. (2006). Succession of bacterial and fungal communities during a traditional pot fermentation of rice vinegar assessed by PCR-mediated denaturing gradient gel electrophoresis *Int J Food Microbiol,* **109**:79–89.
8. Wu, J., Gullo, M., Chen, F. and Giudici, P. (2010). Diversity of Acetobacter pasteurianus strains isolated from Solid-State fermentation of cereal vinegars. *Current Microbiology,* **60**: 280–286.
9. Joshi, V.K., Lal, B.B. and Kakkar, K.S. (2002).Updating the technique of apple chops making and its utilization. *Beverage and Food World,* **17**(1): 21–24.
10. Gullo, M. and Giudici, P. (2008). Acetic acid bacteria in traditional balsamic vinegar: Phenotypic traits relevant for starter cultures selection. *International Journal of Food Microbiology,* **125**:46–53.
11. Du Toit, W.J and Pretorius, I.S. (2002). The occurrence, control and esoteric effect of acetic acid bacteria in winemaking. *International Journal of Food Microbiology,* **52**: 155–179.
12. Yamada, Y. and Yukphan, P. (2008). Genera and species in acetic acid bacteria. *International Journal of Food Microbiology,* **125**:15 –24.
13. Adams, M. R., and Moss, M. O. (2000).Food microbiology. Royal Society of Chemistry, Cambridge **2**225–290.
14. Guizani, N., and Mothershaw, A. (2006). Fermentation: general principles. In: Handbook of Food Science, Technology, and Engineering, Hui, Y. H., eds. Taylor & Francis, London ***63***: 61–28.
15. O'Toole, D. K., and Lee, Y. K. (2003).Fermented foods. In: Microbial Biotechnology: Principles and Applications, Lee, Y. K., ed. World Scientific, Hackensack, NJ 201–256.
16. Brul S and Coote P. (1999). Preservative agents in foods: mode of action and microbial resistance mechanism. *International Journal of Food Microbiology,* **50**:1–17.
17. Kalaba,V., Marjanovićbalaban, Ž. and Kalab, D.(2019). Antibacterial Activity of Domestic Apple Cider Vinegar.*AGROFOR International Journal,* **4**(1): 24–33.
18. Tumane P.M, Sarkar S, Wasnik D.D. and Kolte N.A (2018).Production of vinegar from pineapple peels using Acetobacter species isolated from soil sample and its antibacterial activity. *International Journal of Life Sciences,* **6** (4):948-956
19. Chan, E.W., Fong, C.H., Kang, K.X., Chong, H.H. and Chong, H.H. (2012). Potent antibacterial activity of wood vinegar from Matang mangroves, *Malaysia.SME/G LOMIS Electron Journal,***10**: 10–15.
20. Chang J and Fang T. J. (2007). Survival of Escherichia coli O157:H7 and Salmonella entericaserovarstyphimurium in iceberg lettuce and the antimicrobial effect of rice vinegar against *E. coli O157:H7. Food Microbiology,***24**:745–51.
21. Hassan, R., El-Kadi, S., and Sand. M. (2015).Effect of some organic acids on some fungal growth and their toxins production. *International Journal of Advances in Biology,***2**: 1–11
22. Velmurugan, N., Han, S.S. and Lee, Y.S. (2009). Antifungal activity of neutralized wood vinegar with water extracts of Pinusdensiflora and Quercusserrata saw dusts *International Journal of Environmental Resources*, **3**:167–176.
23. Baimark, Y. and Niamsa, N. (2009) Study on wood vinegars for use as coagulating and antifungal agents on the production of natural rubber sheets. *Biomass Bioenergy,***33**: 994–998
24. Ma, X.H. Wei, Q., Zhang, S.S., Shi, L., Zhao, Z. (2011). Isolation and bioactivities of organic acids and phenols from walnut shell pyroligneous acid. *Journal of Analysis of Applied Pyrolysis,* **91**: 338–343.
25. Hwang, Y.H.; Matsushita, Y.I.; Sugamoto, K. and Matsui, T. (2005).Antimicrobial effect of the wood vinegar from Cryptomeria japonica Sapwood on Plant Pathogenic Microorganisms. *Journal Microbiology and Biotechnology,***15**: 1106–1109
26. Russell, A.D, Tattawasart, U., Maillard, J. andFurr JR. (1998). Possible Link between Bacterial Resistance and Use of Antibiotics and Biocides. *Journal of Antimicrobial Agents and Chemotherapy,* **42**(8):2151–2155.
27. Yang, J., Yang, C., Liang, M., Gao, Z., Wu, Y., and Chuang, L (2016). Chemical Composition, Antioxidant, and Antibacterial Activity of Wood Vinegar from Litchi chinensis. *Molecules,* **21**: 1150
28. Rauha, J.P, Remes, S., Heinonen, M., Hopia, A., Kahk¨onen, M., Kujala, T., Pihlaja, K., Vuorela, H. and Vuorela, P. (2000). Antimicrobial effect of Finnish plant extracts containing flavonoids and

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DOI: http://doi.org/10.5281/zenodo.5716127

otherphenolic compounds. *International Journal of Food Microbiology,* **56**:3–12.

29. Kang, H. C., Park, Y. H. and Go,S. J. (2003). Growth inhibition of a phytopathogenic fungus, Colletotrichum species by acetic acid. *Microbiology Resources,***158**: 321–326
30. Hirshfield, I. N., Terzulli, S., and O'Byrne, C. (2003). Weak organic acids: A panoply of effects on bacteria. *Science Progress,***86**: 245–269
31. Chen, H. Y., Chen, T., Giudici, P. and Chen, F. S. (2016). Vinegar functions on health: Constituents, sources, and formation mechanisms *Comprehensive Reviews in Food Science and Food Safety,* **15**: 1124–1138.
32. Russell, J. B., Diez-Gonzalez, F. (1997). The effects of fermentation acids on bacterial growth. *Advances in Microbial Physiology*, 39: 205–234.
33. Lynch,K.M., Zanini, E., Wilkison, S., Daenen, L. and Arendt, E.K.(2019). Physiology of acetic bacteria and their role in vinegar and fermented brevages. *Comprehensive review in food science and food safety,***13**:587-625
34. Pelaez, A. M. L.; Catano, C. A. S. Yepes,E. A. Q. Villarroel, R. R. G., Antoni, G. L. D. and. Giannuzzi, L. (2012). Inhibitory activity of lactic and acetic acid on *Aspergillus flavus* growth for food preservation. *Food Control,***24**: 177–183.
35. Budak, N., ,Atif, A.C.,Annel, S. Greene, K,and.Guzel-Seydim, Z.B. (2014). Functional Properties of Vinegar. *Journal of Food Science,***79**:757 –763
36. Higgins, C. and F. Brinkhaus, (1999). Efficacy of several organic acids against molds. *Journal of Applied Poultry Resources,* **8**: 480–487.